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IN SILICO ANALYSIS OF ANTIBACTERIAL ACTIVITY OF FATTY ACIDS IN Swietenia humilis Zucc. SEED EXTRACT AGAINST Staphylococcus aureus sortase A ENZYME

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ABSTRACT

This study utilised molecular docking to predict the binding affinity of various fatty acids (FAs) found in *Swietenia humilis* to the sortase A (SrtA) protein target from *Staphylococcus aureus*. Binding energies, measured in kcal/mol, indicated the strength and stability of ligand-protein interactions, with lower values signifying stronger binding. The binding affinities of eight FAs as the active constituents in the *n*-hexane extract of *S. humilis* and the positive control, gentamicin, were compared to assess their theoretical antibacterial activity. Palmitoleic acid exhibited the strongest binding affinity (-5.6 kcal/mol) among the FAs, suggesting the highest potential antibacterial activity, followed by linoleic, palmitic, linolenic, arachidic, tricosanoic, stearic, and oleic acids in decreasing order of affinity. Despite having weaker binding energies than gentamicin, a common gram-positive inhibitor from aminoglycoside derivative, FAs showed multiple hydrogen bonds and van der Waals interactions with key residues like ARG^{197}, VAL^{168}, VAL^{166}, and ILE^{182}, contributing to their binding stability. Palmitoleic acid formed multiple hydrogen bonds (ARG^{197} and GLY^{119}) and significant van der Waals interactions, highlighting its strong theoretical binding. Stearic and oleic acids, although having higher binding energies, also formed critical hydrogen bonds, suggesting moderate potential activity. Despite fewer interaction points, Gentamicin's single hydrogen bond suggests a particular binding site, which may result in high antibacterial activity. The study indicated that FAs like palmitoleic and oleic acid show substantial potential as supplementary antibacterial agents, especially in combating antibiotic resistance. This finding can pave a path for drug design and development to address the *S. aureus's* resistance.

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INTRODUCTION

The resistance of pathological organisms such as bacteria to medical treatments has become a global concern in managing infectious diseases. [1]. *Staphylococcus aureus,* for instance, exemplifies drug-resistant issues due to gene mutations and drug inactivation. [2]. Studies have documented *S. aureus's* resistance to multiple antibiotics, including penicillin. [3], methicillin [4], and vancomycin [5], as well as more recent drugs like daptomycin [6] and linezolid [7]. This resistance leads to ineffective treatments, increased risk of infection spread, disability, and mortality. *S. aureus* is recognised as a pathogenic agent responsible for a range of diseases, from minor skin infections to life-threatening conditions such as pneumonia and meningitis [8].

Various mechanisms contribute to the resistance of *S. aureus* to synthetic antibiotics, including deactivation of the antibiotic (e.g., aminoglycoside-modifying enzymes and penicillinase) [9], modification of the target site reducing antibiotic affinity (penicillin-binding protein 2a in methicillin-resistant *S. aureus* and D-Ala-D-Lac in peptidoglycan precursors of vancomycin-resistant strains) [7, 10], sequestration of the antibiotic (for daptomycin and vancomycin) [11], and efflux systems (for fluoroquinolones and tetracycline) [7]. However, natural products are believed to deactivate microbial activities through multiple approaches. [12-14]. For instance, curcumin, a phenolic compound, has shown inhibitory effects against *S. aureus* by directly binding to the peptidoglycan of the cell wall and damaging RNA involved in protein synthesis. [15]. Additionally, active phytoconstituents can target diverse components of bacteria, including morphology, bacterial cytomembrane, metabolic pathways, nucleic acid formation, and bacterial biofilm. [3].

The emergence of antibiotic resistance encourages the exploration of alternative treatments. Targeting sortase transpeptidase enzymes of *S. aureus*, one of the alternative approaches to minimise drug resistance, offers a compelling approach to combat infections. [16, 17]. Sortase enzymes, such as sortase A (SrtA), are essential for the bacteria to anchor virulence factors to their cell wall and form pili. [18], which are crucial for adhesion and infection establishment [19, 20]. By inhibiting these enzymes, we can effectively disarm the bacteria. [21], reducing their virulence and ability to cause disease [22]Without directly killing them or inhibiting their growth [23]. This indirect approach does not exert the same selective pressure as traditional antibiotics, significantly lowering the likelihood of resistance development. [24]. Additionally, targeting virulence factors may preserve the host's beneficial microbiota, promoting a more balanced and less disruptive treatment option.

Fatty acids (FAs) are well-recognised for their antimicrobial properties, particularly medium- and long-chain FAs [25, 26]. Compounds such as palmitic acid **1**, palmitoleic acid **2**, stearic acid **3**, oleic acid **4**, linoleic acid **5**, linolenic acid **6**, arachidic acid **7**, and tricosanoic acid **8** exhibit various biological activities, including antibacterial effects [25, 27-29]. In our previous study, the

hexane extract of *Swietenia humilis*, which contains these FAs, showed potential anti-*S. Aureus* activity, with inhibition zone diameters ranging from 10 to 17 mm [30]. The extract's composition includes 15.49% of **1**, 0.28% of **2**, 14.04% of **3**, 37.02% of **4**, 24.70% of **5**, 7.33% of **6**, 0.84% of **7**, and 0.33% of **8**.

A current review has highlighted the possible mechanisms of FAs' action, such as inhibition of DNA/RNA duplication, cell wall synthesis, protein synthesis, interception of the cytoplasmic membrane, and inhibition of bacterial metabolism. [28]. Additionally, non-traditional mechanisms were emphasised, including inhibition of horizontal gene transfer, quorum sensing, and antibiotic efflux pumps. [28]. Despite this potential, more comprehensive studies need to focus on the antibacterial activity of these FAs against *S. aureus* sortase A. Existing research often provides fragmented insights, lacking a deep exploration of their molecular interactions with bacterial SrtA targets.

Molecular docking is a powerful computational tool that predicts the interaction between small molecules, such as FAs and target proteins. [31, 32]. It provides insights into compounds' binding affinity and specificity, helping elucidate their potential mechanisms of action. [33]. By simulating the molecular interactions between FAs and bacterial SrtA, docking studies can identify promising antibacterial agents and guide the design of more effective therapeutics. [34-36]. Addressing the current gaps in research through molecular docking studies will enhance our understanding of the antibacterial mechanisms of FAs and their potential role in combating antibiotic-resistant strains of *S. aureus.* [37]. This work will lay a foundation for future experimental and computational studies, ultimately contributing to developing novel antibacterial therapies.

METHODS

1. Materials

1.1. Software

The in-silico preparation and experiment were carried out using the following software due to their well-established performance: ChemDraw 13.0 (https://perkinelinformatics.com/products/research/chemdraw) for creating 2D structures. [38], HyperChem 8.0 (www.hypercubeusa.com) for optimising the 3D structures of ligands [39], PyRx 8.0 (https://pyrx.sourceforge.io) for simulating the molecular docking [40], Biodiscovery Studio 12 (https://discover.3ds.com) for molecule preparation and docking output visualisation [41], and Open Babel 3.1.1 (https://openbabel.org) for converting the data files [42].

1.2. Hardware

The Dell Latitude E7470 computer with Intel Core i7 vPro was employed to run the study, ensuring adequate computational power (4 cores, 8GB of RAM, a 128GB SSD), memory, and data processing capabilities.

2. Method

2.1. Protein preparation

Due to their reliable accuracy, the molecular docking study was conducted using AutoDock Vina integrated within PyRx 8.0 software. The 3D crystal structure of sortase A (SrtA) protein from *S. aureus* (PDB ID: 2kid) was obtained from the Protein Data

Bank (https://www.rcsb.org/structure/2kid) [43] and prepared in Biodiscovery Studio 12. All water molecules and heteroatoms were removed using the following steps: press Ctrl+H to select heteroatoms and water, press Delete on the keyboard and save the file in PDB format. For accurate complex geometry and binding energy estimation, the Gasteiger charges were added by navigating to Chemistry, then Add H Polar, navigating to Edit, selecting Charges, then Compute Gasteiger, and saving in PDB format. The docking simulation was performed using the default active site, with optimised active sites expressed in a grid box coordinate of x = -4.885; y = 0.548; z = 7.232, obtained by defining the binding site of the native ligand of the protein visualised in the Discovery Studio software.

2.2. Ligand preparation

The 3D structures of the FA ligands were created in HyperChem 8.0 and optimised using the semiempirical AM1 method, a reliable method for 3D modelling of organic molecules, in default mode. The molecules presented in Figure 1 include palmitic acid 1, palmitoleic acid 2, stearic acid 3, oleic acid 4, linoleic acid 5, linolenic acid 6, arachidic acid 7, and tricosanoic acid 8. The 3D molecular structure of gentamicin, used as the positive control, was obtained from the Zinc15 database (ZINC8143541 (Gentamicin) (docking.org)) [44] and then optimised in the HyperChem software. Gentamicin was chosen as the positive control due to its broad-spectrum antibacterial activity, particularly against *S. aureus* [45].

Each structure was created using the following steps: in HyperChem 8.0, go to the Build menu and select Build/Edit to enter the molecule-building mode, use the drawing tools to create the carbon backbone of the FA and add hydrogen atoms by selecting the Element tool and clicking on each carbon to complete the valence, go to the Build menu and select Model Build to automatically correct any structural irregularities, and use the Geometry Optimization tool to refine the 3D structure further. Structure optimisation was achieved by going to the Setup menu, choosing Semi-Empirical, selecting AM1 as the method, ensuring that the calculation is set to Default mode, and clicking OK to confirm the setup. The following steps describe the geometry optimisation: go to the Compute menu and select Geometry Optimization, set default settings, then click OK to start the optimisation process; once the optimisation is complete, go to the File menu and select Save As the optimised structure in the PDB format. Gasteiger charges were assigned to all ligands to achieve accurate binding energy. Assigning these charges ensures that the docking simulations are based on accurate charges, essential for reliable modelling of electrostatic interactions, molecular conformations, and binding affinities [46].

2.3. Molecular docking setup

PyRx integrated with AutoDock Vina simplifies the molecular docking process [47], facilitating the preparation, execution, and analysis of docking simulations. In the PyRx platform, the prepared protein was loaded into the program by right-clicking the

file name, selecting Autodock, and choosing "Make Macromolecule". The ligand was then opened via the following steps: In the control panel, open Babel for file format conversion and insert a new item, select the ligand file, then right-click the ligand name in the control box and select 'Minimize Selected', convert the ligand to AutoDock ligand format (pdbqt), and select the ligand in the Ligands layer by holding Ctrl and double-clicking the ligand folder on the left. The molecular docking simulation was then generated using the following steps to enable docking simulation to occur on the platform: click on Vina Wizard, then Start, select the ligand by clicking its name in the Ligands folder and click Forward; the main screen will show the protein with the docking location box, click Forward, save the resulting data table as a CSV file for analysis in MS Excel. To view the output, the following steps were taken: in PyRx, click the AutoDock button on the top left, double-click the macromolecule file name in the Macromolecule folder, right-click the ligand name and choose Display, save the conformation by right-clicking the file name and selecting Save as PDB. The software ranked the obtained conformation from the most recommended 3D structure to the least option.

2.4. Data analysis

The interactions between ligands and receptors were visualised as the following procedures: open the ligand file with the best conformation in Biodiscovery Studio, copy the ligand structure and paste it into the previously prepared protein file, navigate to Receptor-Ligands Interactions, click View Interaction, and define the receptor and ligands by selecting the ligand. To view protein-ligand interactions in 2D, choose Show 2D Diagram. To view the interaction data table, right-click on the model panel, select View, then Data Table and See Non-Bond. Use Show Type of Interaction or Show Distance to measure the distance between ligand atoms and amino acid residues for interaction details. AutoDock Vina uses an empirical scoring function to estimate the binding energy (ΔG) using the following components: intermolecular energy, internal energy of the ligand, desolvation energy, and torsional free energy. The binding affinities of the FAs were compared with those of Gentamicin to evaluate the potential effectiveness of these compounds as antimicrobial agents.

RESULTS AND DISCUSSION

1. Binding affinity results

The molecular docking study provides a theoretical framework for predicting the binding affinity of various compounds to the *S. aureus* SrtA protein target. SrtA is located on the extracellular side of the membrane and has three conserved amino acid residues within the active sites: His120, Cys184, and Arg197 [43]. Binding energy, measured in kcal/mol, indicates the strength and stability of the interaction between the ligand (FAs and gentamicin) and the protein, with lower values corresponding to stronger and potentially more effective interactions. This discussion compares the binding energies of eight FAs and the positive control, gentamicin, to evaluate their theoretical antibacterial activity.

Palmitic acid 1 showed a moderate binding affinity with SrtA compared to the

positive control, indicating a potentially effective interaction. Palmitoleic acid 2 exhibited the strongest binding affinity among the FAs studied, suggesting it may have the highest potential antibacterial activity within this group. Palmitoleic acid has been believed to protect human skin from the production of virulence determinants by *S. aureus* and from the induction of antimicrobial resistance [48]. By reducing the bacteria's ability to express virulence factors and develop resistance, these fatty acids help maintain the effectiveness of antibacterial treatments. Stearic acid 3 had a slightly weaker binding affinity than palmitic 1 and palmitoleic 2 acids, implying a moderately effective interaction.

Furthermore, oleic acid 4 demonstrated the weakest binding affinity among the FAs, indicating a less effective interaction with the target protein. While oleic acid engages in van der Waals interactions with several amino acid residues (e.g., VAL^{168}, VAL^{166}, ILE^{182}), these interactions alone may not be sufficient to compensate for the weaker hydrogen bonding, leading to a less stable overall binding affinity. Linoleic acid 5, with a binding energy similar to palmitic acid 1, also suggests a moderate binding affinity and potential antibacterial activity. Linolenic acid 6 had a slightly stronger binding affinity than stearic acid 3 but weaker than palmitoleic acid, suggesting a fairly effective interaction. Multiple double bonds in linolenic acid increase its flexibility, allowing it to adapt better and fit into the binding site of the target protein. This increased flexibility can facilitate more effective interactions with the active site residues of the SrtA protein. Arachidic acid 7 showed a comparable binding affinity to tricosanoic acid 8, suggesting moderate interaction strength for both.

All the FAs exhibited weaker binding affinities than gentamicin, suggesting that while they may possess antibacterial properties, they are likely less potent than gentamicin. Palmitoleic acid 2, which has the strongest binding affinity among the FAs, still falls short of gentamicin's binding energy by 1.4 kcal/mol. Indeed, palmitoleic acid 2 is the major endogenous antibacterial agent against *S. aureus* found on the skin of mammalian species [49], [50].

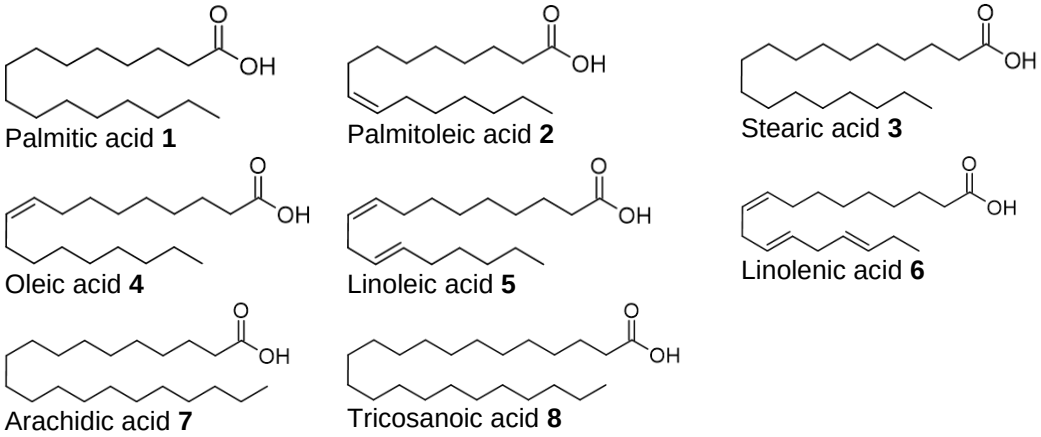


Figure 1. The 2D molecular structures of FAs detected in the *n*-hexane extract of *S. humilis* seeds [30] for this study

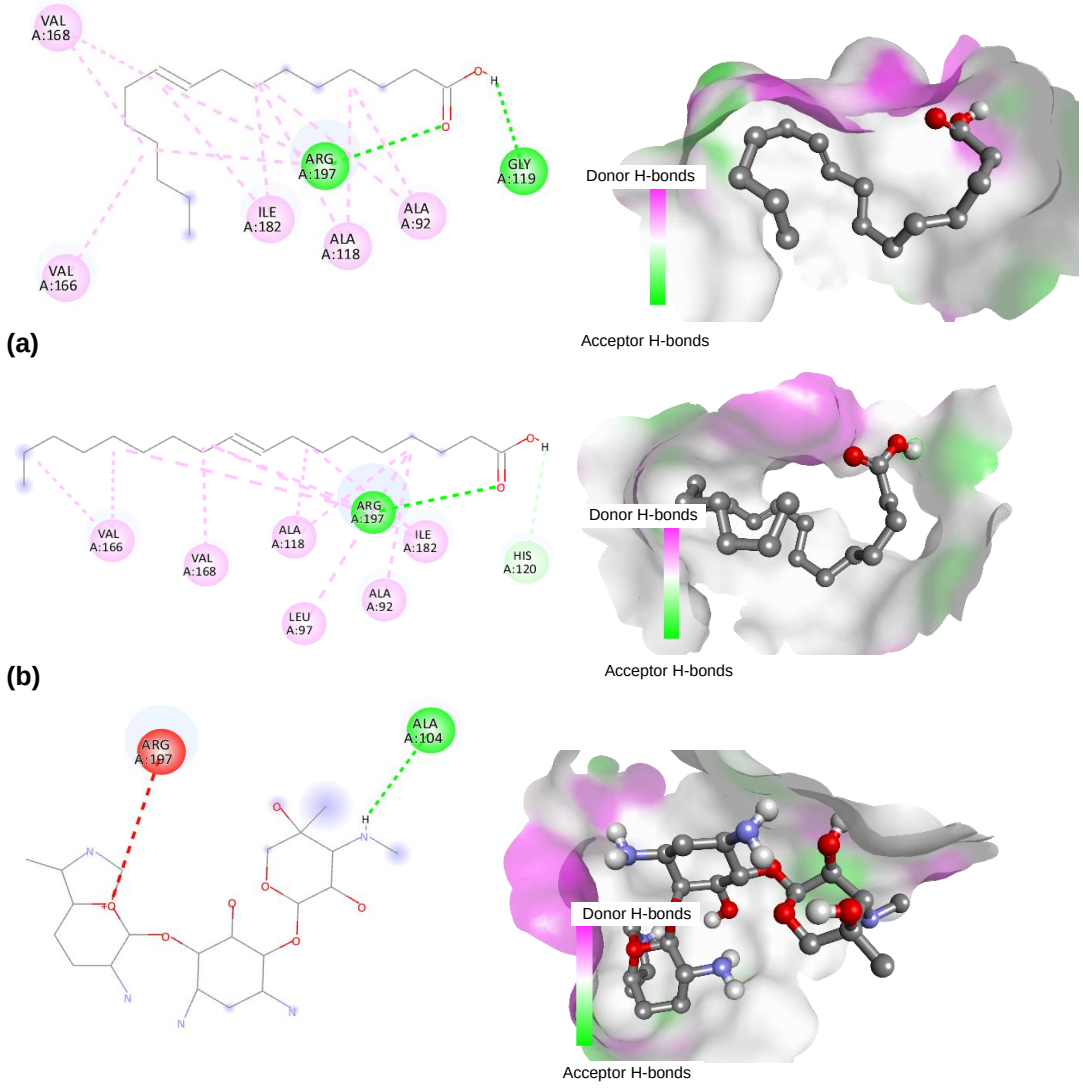
Table 1. Observed data from the virtual screening interaction between ligands and protein in this study

No	Compound	Observation		
		Binding affinities (kcal/mol)	Type of interaction	Amino acid residues
1	Palmitic acid 1	-5.5	van der Waals	ARG^{197}, VAL^{168}, VAL^{166}, ALA^{104}, ALA^{92}, ALA^{118}, LEU^{169}
			Hydrogen bond	ILE^{182}, GLY^{119}, HIS^{120}
2	Palmitoleic acid 2	-5.6	van der Waals	VAL^{168}, VAL^{166}, ILE^{182}, ALA^{92}, ALA^{118}
			Hydrogen bond	ARG^{197}, GLY^{119}
3	Stearic acid 3	-5.1	van der Waals	ARG^{197}, VAL^{168}, VAL^{166}, ILE^{182}, ALA^{118}, LEU^{169}, ALA^{92}, ALA^{104}
			Hydrogen bond	GLY^{119}, HIS^{120}
4	Oleic acid 4	-4.9	van der Waals	VAL^{168}, VAL^{166}, ILE^{182}, ALA^{118}, LEU^{97}, ALA^{92}
			Hydrogen bond	ARG^{197}, HIS^{120}
5	Linoleic acid 5	-5.5	van der Waals	VAL^{166}, ARG^{197}, VAL^{168}, LEU^{169}, ALA^{104}, ILE^{182}, ALA^{118}, ALA^{92}
			Hydrogen bond	GLY^{119}, HIS^{120}
6	Linolenic acid 6	-5.4	van der Waals	ALA^{118}, ILE^{182}, ARG^{197}, VAL^{168}, VAL^{166}, ALA^{92}
			Hydrogen bond	HIS^{120}, CYS^{184}
7	Arachidic acid 7	-5.3	van der Waals	VAL^{166}, ARG^{197}, VAL^{168}, ALA^{104}, ILE^{182}, ALA^{118}, ALA^{92}
			Hydrogen bond	GLY^{119}, HIS^{120}
8	Tricosanoic acid 8	-5.3	van der Waals	ALA^{104}, VAL^{168}, ALA^{118}, VAL^{166}, LEU^{97}, ALA^{92}
			Hydrogen bond	GLY^{119}, ARG^{197}, ILE^{182}
9	Gentamicin (positive control)	-7	Hydrogen bond	ALA^{104}

2. Interaction analysis

The number of hydrogen bonds and their respective distances indicated varying degrees of interaction with the SrtA protein. For example, palmitoleic acid 2 formed multiple hydrogen bonds with key amino acid residues (ARG^{197} twice at 2.96 and 3.08 Å, and GLY^{119} at 1.96 Å), contributing to a stable binding interaction. These hydrogen bonds suggest a stable binding interaction, indicated by the lowest binding energy among the other FAs, as multiple bonds at close distances create a robust attachment to the protein's active site. Similarly, oleic acid **4** formed two strong hydrogen bonds with ARG^{197} (2.33 and 2.26 Å), suggesting a significant binding affinity. The hydrogen bonding with GLY^{119} manifested the essential role of this amino acid, shown by the second-ranked score of palmitic acid **1** with hydrogen bonds to ILE^{182}, GLY^{119}, and HIS^{120}.

All FAs engaged in van der Waals interactions with residues such as ARG^{197}, VAL^{168}, VAL^{166}, and ILE^{182}, contributing to the overall binding stability. These interactions supplemented the hydrogen bonds and enhanced the binding affinity. The van der Waals interactions, along with hydrogen bonds, play an integral role in the binding affinity of FAs to the SrtA enzyme by providing additional stabilisation, complementarity, and cumulative binding energy, which are essential for the effective inhibition of the enzyme's activity.



Note:
(--) : van der Waals interaction
(--) : hydrogen bond
(--) : unfavoured interaction

Figure 2. The 2D (left) and 3D (right) representation of amino residues of SrtA protein of *S. aureus* with selected compounds of (**a**) palmitoleic acid **2**, (**b**) oleic acid **4**, and (**c**) gentamicin

2. Interaction analysis

With multiple hydrogen bonds and significant van der Waals interactions, palmitoleic acid **2** (-5.6 kcal/mol) showed the strongest theoretical binding among the FAs, indicating high potential antibacterial activity. The findings on palmitoleic acid's binding affinities and interactions align well with experimental data from other studies, showing its significant antibacterial activity. For instance, studies have shown that palmitoleic acid can interfere with the synthesis of peptidoglycan, an essential component of the bacterial cell wall, thereby inhibiting bacterial growth and survival [51, 52].

Despite weaker overall binding energies of stearic acid **3** (-5.1 kcal/mol) and oleic acid **4** (-4.9 kcal/mol), their hydrogen

bond interactions suggested moderate potential activity. Both compounds formed critical hydrogen bonds with GLY^{119} HIS^{120}, and ARG^{197,} respectively. These findings emphasised the vital role of both carbonyl and hydroxyl groups. Moderate binding affinities do not preclude synergistic effects. When combined with other antibacterial compounds, stearic and oleic acids might produce synergistic effects that enhance overall antibacterial activity, even if their contributions are modest.

The single hydrogen bond interaction in gentamicin indicated a particular binding site bond with ALA^{104} by the secondary amine group, which might translate to high antibacterial activity despite fewer interaction points. ALA^{104} has been recognised as the prominent constituent of the hydrophobic pocket of the enzyme active site. Interfering this amino acid residue resulted in the disrupted SrtA transpeptidase activity. [35]. Interestingly, none of the hydrogen bonds with ALA^{104} residue were found in the interaction between FAs and the enzyme's active site, suggesting the crucial role of bonding with ALA104 in effectively deactivating the enzyme activity. This is likely due to the lack of electronegative groups, *i.e.* amine and hydroxyl, on the long-chain carbon tail of the FAs. The spatial orientation of the ALA104 residue within the active site might not favour the formation of hydrogen bonds with the FAs due to steric hindrance or distance constraints. In fatty acid interactions, the absence of hydrogen bonds with the ALA104 residue could lead to reduced binding affinity and stability. However, the antibacterial activity of FAs can still be significant through alternative binding interactions and other mechanisms like membrane disruption.

The comparison based on intermolecular interactions suggests that while gentamicin exhibited the strongest binding affinity due to its significantly lower binding energy, FAs like palmitoleic acid and oleic acid showed substantial potential due to their multiple hydrogen bonds and van der Waals interactions with the essential amino acids of the active site of SrtA. These FAs might serve as supplementary antibacterial agents, especially in resistance, where combining agents can be beneficial. Future studies such as surface plasmon resonance or isothermal titration calorimetry can experimentally quantify the binding affinities of the FAs with SrtA in a more dynamic setting.

The analysis of binding affinities and intermolecular interactions reveals a pattern where monounsaturated fatty acids (MUFAs), particularly palmitoleic acid, show the highest binding affinity among the FAs studied, likely due to the presence of a double bond enhancing flexibility and interaction potential. The single, double bond in MUFAs like palmitoleic acid allows the molecule to maintain flexibility while being more rigid than fully saturated fatty acids. This balance between rigidity and flexibility enables the fatty acid to adapt its conformation to fit snugly into the enzyme's active site. Polyunsaturated (PUFAs) and saturated fatty acids exhibit moderate binding affinities with varying interaction patterns. The multiple hydrogen bonds and extensive van der Waals interactions in FAs suggest their

potential as supplementary antibacterial agents. PUFAs have multiple double bonds, which can introduce too much flexibility and result in less stable interactions with the binding site. This can lead to weaker binding affinities compared to MUFAs.

3. Theoretical implications of the findings

Unsaturated FAs have previously been documented to exhibit anti-S directly. *Aureus* activity. The antibacterial activities of natural seed oil of apricot, date, grape, and black seeds were linked to the increased level of linoleic acid **5** [26]. This finding was confirmed by comparing the evaluation of each seed oil treatment demonstrating weaker or no antibacterial activity. Indeed, palmitoleic acid **2** and linoleic acid **5** can alter the peptidoglycan synthesis genes, inhibiting the cell wall biosynthesis of *S. aureus.* [28]. Moreover, linoleic acid **5** was also reported to change the gene expressions of glycolytic and fermentative metabolic pathways, leading to a shortage of energy production in *S. aureus.* [53]. By altering gene expression, linoleic acid can reduce the production of virulence factors such as toxins, enzymes, and adhesion molecules, decreasing the pathogenicity of the bacteria. [54].

Even though the study of the effect of FAs on the non-traditional inhibitory mode of action against *S. aureus* SrtA remains restricted, the latest investigation through high-throughput virtual screening (HTVS) on unsaturated FAs has confirmed the inhibitory properties against SrtA from another *Staphylococcus* species, *S. mutans* [55]. The work has identified several unsaturated FAs, including linolenic acid derivatives, with strong binding affinities to SrtA. [55] They are ranging from 5.9 kcal/mol to 8.9 kcal/mol. The interactions were primarily stabilised by hydrogen bonds and hydrophobic interactions linked to carbonyl and hydroxyl groups and the long-chain backbone, involving key residues like Thr^{586}, Val^{587}, and Phe^{656}. These interactions suggest that the unsaturated FAs with extended hydroxyl groups on the backbone carbon chain can effectively bind to and inhibit the activity of SrtA. In both *S. aureus* and *S. mutans*, unsaturated FAs like palmitoleic acid demonstrated significant binding affinity, which suggests that the inhibitory properties of these FAs against SrtA are consistent across different *Staphylococcus* species. This indicates a potential broad-spectrum application of these FAs as antibacterial agents targeting SrtA, enhancing their utility in combating infections caused by different strains of *Staphylococcus*.

Saturated FAs were also believed to exert bactericidal activity against *S. aureus* through direct action. Two major lipid components of wings of cicadas and dragonflies, palmitic **1** and stearic acids **3**, were exhibited 95.4 % and 73% inhibition against *S. aureus* cells, respectively [25]. In line with this report, we also found that palmitic acid 1 possessed better binding affinity (5.5 kcal/mol) than stearic acid **3** (5.1 kcal/mol)**.** The shorter chain of palmitic acid can provide greater flexibility, enabling it to adopt a conformation that maximises interactions with the enzyme's active site. Stearic acid, with its longer chain, might face more conformational constraints, limiting its ability to interact optimally. These two saturated acids**, 1 and 3,** inhibited quorum

sensing of a gram-negative species of *Vibrio harveyi* [56].

Saturated FAs were also believed to exert bactericidal activity against *S. aureus* through direct action. Two major lipid components of the wings of cicadas and dragonflies, palmitic acid 1 and stearic acid 3 exhibited 95.4% and 73% inhibition against *S. aureus* cells, respectively [25]. In line with this report, palmitic acid 1 was found to have a better binding affinity (-5.5 kcal/mol) than stearic acid 3 (-5.1 kcal/mol). The shorter chain of palmitic acid provides greater flexibility, enabling it to adopt a conformation that maximises interactions with the enzyme's active site. Stearic acid, with its longer chain, might face more conformational constraints, limiting its ability to interact optimally. Both acids were involved in inhibiting quorum sensing of a gram-negative species of *Vibrio harveyi* [56].

Furthermore, some FAs disrupt the virulence and interaction of *S. aureus* sortase enzymes with the host extracellular matrix. The palmitoleic **2** and linoleic **5** demonstrated protecting effects on human skin from producing *S. aureus* virulence determinants and the induction of antimicrobial resistance. [57]. The clinical implications of disrupting the virulence factors of *S. aureus* with fatty acids are substantial. These include reducing the severity of infections, enhancing the efficacy of antibiotic treatments, improving patient outcomes by minimising tissue damage and inflammatory responses, and offering prophylactic options for high-risk patients. Additionally, this approach can lead to the development of novel therapeutic strategies that focus on attenuating bacterial virulence, ultimately contributing to better management and control of *S. aureus* infections.

Given the absence of information on molecular-scaled interactions, we here displayed the possible interactions of naturally occurring FAs in inhibiting *S. aureus* SrtA by two main intermolecular forces with various active pockets of the enzyme, including guanidinium moiety of Arg^{197}, two putative catalytic amino acid residues Arg^{197} and $Cys^{184.}$ [58]. The interactions with Arg^{197} and Cys^{184} are critical for the inhibitory activity of FAs against SrtA. Arg^{197} is essential for substrate stabilisation and catalytic activity. [59], while Cys^{184} plays a crucial nucleophilic role in the transpeptidation reaction [60]. FAs that can effectively interact with these residues disrupt the enzyme's function, leading to potent inhibition and reduced bacterial virulence. Modifying the carbon backbone chain of FAs with electron-donating groups could potentially enhance their inhibitory efficacy against the pathogenic SrtA enzyme.

CONCLUSION

As a computational study to establish a theoretical foundation for the development of novel antibacterial therapies, this in-silico study indicates that while FAs such as palmitoleic acid, palmitic acid, and linoleic acid showed potential antibacterial activity against *S. aureus* by binding to the SrtA protein, their theoretical effectiveness is lower compared to the positive control, gentamicin. This implies the importance of the different modes of action. These findings highlight the potential of certain FAs as supplementary antibacterial agents for food

and drug products but also underscore the superior binding and likely higher antibacterial efficacy of gentamicin. Further experimental validation, including in vitro or in vivo studies, is essential to confirm these theoretical predictions and to fully understand the antibacterial mechanisms of these FAs against *S. aureus.*

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